

Patent Claims

1. Variant of the major allergen Phl p 1 from timothy grass, characterised in that it has an additional Cys residue compared with the wild type.
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2. Allergen variant according to Claim 1, characterised in that the additional Cys residue is located in the carboxyl-terminated region.
- 10 3. Allergen variant according to Claim 1 or 2, characterised in that the additional Cys residue is located in a higher position than amino acid position 140.
- 15 4. Allergen variant according to one or more of Claims Claim 1 to 3, characterised in that the additional Cys residue is located between amino acid positions 230 and 240.
- 20 5. Allergen variant according to one or more of Claims 1 to 4, characterised in that the additional Cys residue originates from an amino acid exchange.
- 25 6. Allergen variant rPhl p 1-A236C according to SEQ ID NO 2 according to one or more of Claims 1 to 5, characterised in that the additional Cys residue has been introduced by exchange of Ala 236.
- 30 7. DNA molecule which encodes for an allergen variant according to one or more of Claims 1 to 6.
8. DNA molecule according to SEQ ID NO 1 which encodes for the allergen variant according to Claim 6.

9. Process for the preparation of a variant of the recombinant major allergen rPhl p 1 according to one or more of Claims 1 to 6, characterised in that, by methods known per se,

5 - a base triplet encoding for a Cys residue is introduced the corresponding gene by insertion or exchange,

10 - the gene modified in this way is overexpressed in a host organism and

 - the allergen variant obtained by overexpression is purified.

15 10. Process for the preparation and purification of a variant of the recombinant major allergen rPhl p 1 according to Claim 9 in soluble form, characterised in that the initially insoluble crude protein is denatured, subsequently renatured by dilution and purified by biochemical purification steps.

20 11. Process for the purification of a variant of the recombinant major allergen rPhl p 1 according to Claim 9 in soluble form, characterised in that, starting from the overexpressed, initially insoluble crude protein provided with an His tag for purification purposes, a plurality of biochemical purification steps, encompassing two-stage metal ion chelate affinity chromatography and the removal of the His tag, are carried out.

25 12. Allergen variant according to one or more of Claims 1 to 6, characterised in that it exists in various fold forms.

30 13. Fold form rPhl p 1-LM of the allergen variant according to one or more of Claims 1 to 6, obtainable by carrying out the following process steps:

 - overexpression of the rPhl p 1 allergen variant provided with an His tag in a host organism,

 - denaturing of the inclusion bodies isolated from the host organism using guanidinium chloride

 - renaturing of the dissolved protein on a chelate affinity chromatogra-

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phy column

- removal of the His tag
- gel filtration
- further chelate affinity chromatography
- isolation of the target protein from the flow-through
- gel filtration.

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14. Fold form rPhl p 1-HM of the allergen variant according to one or more of Claims 1 to 6, obtainable by carrying out the following process steps:

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- overexpression of the rPhl p 1 allergen variant provided with an His tag in a host organism
- denaturing of the inclusion bodies isolated from the host organism using guanidinium chloride
- renaturing of the dissolved protein on a chelate affinity chromatography column
- removal of the His tag
- gel filtration
- further chelate affinity chromatography
- elution of the target protein with an imidazole gradient
- gel filtration.

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15. Allergen variant according to one or more of Claims 1 to 6 and 12 to 14 as medicament.

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16. Use of an allergen variant according to Claim 15 and/or pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios, for the preparation of a medicament for specific immunotherapy of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved.

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17. Pharmaceutical composition comprising an allergen variant according to Claim 15 and/or pharmaceutically usable derivatives thereof,

including mixtures thereof in all ratios, and, if desired, excipients and/or adjuvants.

5 18. Use of an allergen variant according to one or more of Claims 1 to 6
and 12 to 14 and/or derivatives thereof, including mixtures thereof in all
ratios, for the *in vitro* diagnosis of allergies in the triggering of which the
major allergen Phl p 1 from timothy grass is involved.

10 19. Recombinant DNA expression vector containing a DNA molecule
according to Claim 7 or 8 for the treatment of allergies in the triggering
of which the major allergen Phl p 1 from timothy grass is involved, by
immunotherapeutic DNA vaccination.

15 20. Use of the expression vector according to Claim 19 and/or derivatives
thereof, including mixtures thereof in all ratios, for the preparation of a
medicament for the treatment of allergies in the triggering of which the
20 major allergen Phl p 1 from timothy grass is involved, by immunothera-
peutic DNA vaccination.

25 21. Pharmaceutical composition comprising an expression vector accord-
ing to Claim 19 and/or pharmaceutically usable derivatives thereof,
including mixtures thereof in all ratios, and, if desired, excipients and/or
adjuvants, for the treatment of allergies in the triggering of which the
major allergen Phl p 1 from timothy grass is involved, by immunothera-
peutic DNA vaccination.

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